

**AMENDMENTS TO THE SPECIFICATION**

Please replace the paragraph *beginning on page 26, line 37* with the following amended paragraph:

-An alternate method for screening hybridomas for antibody production is as follows. *Pythium insidiosum* is heat-denatured in 0.5 M Tris (pH 7.4) with 10% SDS, 20% glycerol and 5% 2-mercaptoethanol. The denatured antigens are separated by SDS-polyacrylamide gel electrophoresis in a 12-20% (v/v) linear gradient gel with a 4% (v/v) stacking gel. The separated antigens are electrophoretically transferred to Western PVDF membranes at 100 volts for 1.5 hours, then 150 volts for 0.5 hours. The membranes are then blocked overnight in 1% by volume bovine serum albumen in 0.5% ~~Tween-Tris~~ TWEEN TRIS buffered saline (Blocking buffer). The blots are air-dried and stored frozen. Prior to use, the membranes are incubated with bovine serum albumin in Blocking buffer at a range of 1:10 to 1:100 ratio for two hours. Afterwards, the membranes are washed in 0.5% ~~Tween-Tris~~ TWEEN TRIS buffered saline and then incubated with monoclonal antibodies from the various hybridoma clones. The membranes are developed as disclosed in the prior art, e.g., Granstrom et al., J. Vet. Diag. Invest. 5: 88-90 (1993) or *Antibodies, A Laboratory Manual*, eds. Harlow and Lane, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988).-

Please replace the paragraph *beginning on page 34, line 31* with the following amended paragraph:

-The ELISA was carried out as per Mendoza et al. (Mendoza et al., Clin. Diag. Lab. Immunol. 4: 715-718 (1997)). Flat-bottom polystyrene microtiter plates (96-well ~~Immulon2~~ IMMULON2; Dynatech Laboratories Inc., McLean, Virginia) were coated with the antigen prepared as above and incubated overnight at 4° C and then blocked for 1 h at 37° C with 5% gelatin. Dilutions of the sera under investigation were prepared and then added to the coated plates and incubated for 1 h. After several washes, 100 µl of a horseradish peroxidase-conjugated rabbit anti-horse, anti-dog, or anti-cat (heavy and light chains) antibody was added to each well and then incubated at 30° C for 1 h. After incubation, the reaction was stopped with chromogen buffer and color development was recorded in a Dynatech MR 5000 ELISA plate reader at 490 nm. The immunoperoxidase assay performed on sera from two of the dogs was not carried out in our facilities, but was carried out in other laboratories at the request of their owners.-

Please replace the paragraph *beginning on page 53, line 7* with the following amended paragraph:

-To evaluate the different IgG isotypes triggered in the experimentally-induced pythiosis or by

the PIV, and IgG isotype assay is performed. Rabbits are bled before inducing pythiosis, 14 days post-vaccination, and 14 days after the second immunization. The isotype assays measure the total immunoglobulin populations in the rabbit. briefly, 50  $\mu$ l of the PIV (2 mg/ml) is coated on flat-bottomed polystyrene microtiter plates (96-well, ~~Immunolon 2~~ IMMUNOLON2, Dynatech Laboratories, Inc., Virginia) at 4° C for 24 hr. The plates are then reacted against the rabbit sera as per Mendoza et al., Clin. Diagn. Lab. Immunol. 4: 715-718 (1997) followed by reacting with anti-IgG isotypes (IgG<sub>1</sub>, IgG<sub>2</sub>, and IgG<sub>3</sub>) according to the manufacturer's instructions (Accurate Chemicals, New York). In addition, to monitor the Th2 to Th1 switching, IgE levels in all the rabbits are determined. The immunological data provided by the method provides a means for validating the Th2 to Th1 switching hypothesis which can be extrapolated to other infectious diseases of animals. -

Please replace Table 1 *beginning on page 38* with the following amended Table 1:

Table 1. Clinical features of equine cases with pythiosis used in this study and their responses to *Pythium insidiosum*-vaccine

| State   | Age/sex | Lesions                                | Duration of illness | Diagnosis                        | Previous treatments  | Vaccination reaction | Outcome                      |
|---------|---------|--|---------------------|----------------------------------|----------------------|----------------------|------------------------------|
| AR (Gi) | 4 y/F   | Abdomen, 220X220 mm                    | 4 months            | ID, ELISA (+)                    | Surgery, drugs       | Strong, 123 mm       | Cured                        |
| FL (Se) | 13 y/F  | Face, 80X30 mm                         | >4 months           | ID, ELISA (+)                    | Surgery, Drugs       | Mild, 25 mm          | Cured                        |
| FL (Sn) | 12 y/M  | Limb, 150X100 mm                       | > 2 months          | ID (+), clinical                 | Surgery              | Mild, 60 mm          | Cured                        |
| FL (Jo) | 20 y/M  | Limb, 300X150 mm                       | > 2 months          | ID (+), clinical                 | Several surgeries    | Mild, 60 mm          | Cured                        |
| FL (Wa) | 8 y/F   | Limb, 60X50 mm                         | 5-7 days            | ID (+), Clinical                 | Surgical debridement | Strong, 100 mm       | Cured                        |
| FL (Ho) | 5 y/F   | Shoulder & abdomen<br>50X50 & 120X20mm | 15 days             | ELISA (+)                        | Cryosurgery          | Strong, 200 mm       | Cured                        |
| FL (Pe) | 22 y/M  | Limb, 60X40                            | 17 days             | ID, ELISA (+)                    | Surgical debridement | Mild, 30 mm          | Cured                        |
| LA (Re) | 5 y/M   | Limb, 120X100 mm<br>(two lesions)      | 2 months            | ELISA (+)                        | Surgical debridement | Mild, 90 mm          | Cured                        |
| MS (Ba) | 3 y/F   | Limb, 250X250 mm                       | >2 months           | ELISA (+)                        | Topical drugs        | Strong, 150 mm       | Cured                        |
| MS (Pe) | 15 y/F  | Limb, 300X200 mm                       | 4 months            | ELISA (+)                        | Topical drugs        | Strong, 170 mm       | Cured<br>(Vaccine + surgery) |
| MS (Im) | 2 y/M   | Limb, 300X300 mm                       | 4 months            | ELISA (+)                        | Topical drugs        | Strong, 200 mm       | Cured<br>(Vaccine + surgery) |
| MS (So) | 7 y/F   | Limb, 240X240 mm                       | 3 months            | ELISA (+),<br>Histopathology (+) | Topical Drugs        | Strong, 150 mm       | Not cured                    |
| NC (Sa) | 20 y/M  | Inguinal, 60X50 mm<br>(two lesions)    | >2 months           | ID (+), ELISA (+)                | Surgery              | Mild, 40 mm          | Not cured                    |
| NC (Ga) | 14 y/F  | Inguinal 200X150 mm                    | >2 months           | ID (+), ELISA (+)                | Surgery              | Mild, 30 mm          | Not cured                    |
| TN (Re) | 4 y/F   | Abdomen, 200X80 mm                     | 2 months            | ELISA (+)                        | Topical Drugs        | No response          | Not cured                    |
| TX (Ta) | 13 y/M  | Limb, 250X100 mm                       | ~4 months           | ELISA (+)                        | Topical Drugs        | Strong, 200 mm       | Died                         |
| TX (Ah) | 5y/M    | Limb, 280X210 mm                       | 1 month             | Clinical, kunkers<br>ELISA (+)   | Surgery, drugs       | Mild, 30 mm          | Cured                        |
| TX (Co) | 6 y/F   | Limb, 100X80 mm                        | 1 month             | ELISA (+)                        | Topical drugs§       | Weak, 15 mm          | Not cured                    |
| TX (Sn) | 22 y/M  | Mouth, 150X100 mm                      | >2 months           | ELISA (+)<br>Histopathology (+)  | Surgical debridement | Weak, 5 mm           | Not cured                    |